

Amanita wadulawitu (Basidiomycota), a new species from Western Australia, and an expanded description of *A. kalamundae*

Laurton E. McGurk^{1,2,3}, Danielle Giustiniano², Elaine M. Davison^{1,4,5}
and Elizabeth L.J. Watkin²

¹Department of Environment and Agriculture, Curtin University, GPO Box U1987, Perth, Western Australia 6845

²School of Biomedical Sciences, Curtin University, GPO Box U1987, Perth, Western Australia 6845

³Present address: Human Resources, The University of Western Australia, Crawley, Western Australia 6009

⁴Western Australian Herbarium, Department of Parks and Wildlife, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983

⁵Corresponding author, email: e.davison@curtin.edu.au

Abstract

McGurk L.E., Giustiniano, D., Davison, E.M., & Watkin, E.L.J. *Amanita wadulawitu* (Basidiomycota), a new species from Western Australia, and an expanded description of *A. kalamundae*. *Nuytsia* 27: 21–30 (2016). A new species of *Amanita* Pers. is documented from Western Australia. *Amanita wadulawitu* L.E.McGurk, E.M.Davison & E.L.J.Watkin is described from the Perth IBRA subregion. *Amanita kalamundae* O.K.Mill. is redescribed to include additional collections, drawing attention to the presence of clamp connections in the lamellae and at the base of basidia. A BLASTn search has shown that there are no exact matches of the nuclear ribosomal internal transcribed spacer (ITS) region of either species in GenBank.

Introduction

The large, cosmopolitan genus *Amanita* Pers. (Agaricales: Amanitaceae) is a conspicuous part of the fungal flora of the Australian bush. It is divided into two subgenera: subg. *Amanita sensu* Corner & Bas as emended by Bas (1969) and subg. *Lepidella* (E.-J.Gilbert) Vesely as emended by Corner and Bas (1962). The spores of subg. *Amanita* are inamyloid whilst those of subg. *Lepidella* are amyloid. Both subgenera are divided into sections based on the form of the universal veil, position of the primordium, elongation of the stipe and form of the pileus margin (Corner & Bas 1962; Bas 1969). The large sect. *Lepidella* (E.-J.Gilbert) Corner & Bas of subg. *Lepidella* was monographed by Bas (1969). He considered that it was the most primitive section of the genus; most Australian amanitas are in this section. Bas (1969) subdivided sect. *Lepidella* into four subsections which were further subdivided into stirpes (informal groupings of species with similar characters), based on the abundance of inflated cells in the universal veil, their orientation, the presence of clamp connections, pigmentation of the pileus and spore size. Wood (1997) used this system in his taxonomic treatment of *Amanita* spp. in eastern Australia, but Reid (1980), Miller (1991, 1992) and Grgurinovic (1997) did not. Bas' treatment of sect. *Lepidella* has the advantage of providing a workable taxonomic framework until a molecular phylogeny is available.

DNA barcodes have become an important identification tool for all organisms. The nuclear ribosomal internal transcribed spacer (ITS) region has been adopted as the primary fungal barcode marker

(Schoch *et al.* 2012). For the Basidiomycota this had the highest resolving power (0.77) for species discrimination in a comparison of four markers, and a clear barcode gap separating intra- and inter-specific samples. As far as we are aware, the barcode gap has not been established for the ITS region for species of *Amanita*. However Hughes *et al.* (2009), in a study of agarics from the Great Smokey Mountains National Park, have suggested that divergence of 3% or less captured 99% of heterozygotes. We have used this percentage in our study until a better estimate is available.

This paper is one of a series that aims to better characterise *Amanita* species from the south-west of Western Australia. A new species is described from sect. *Lepidella*. During our investigations into local *Amanita* spp. we found that the protologue of *A. kalamundae* O.K.Mill. (MycBank number MB560219) failed to mention that clamp connections are present in the lamellae and at the base of basidia (Miller 1991); this is an important character in the genus. We provide therefore an amended and expanded description of this species. ITS sequences have been generated for both species.

Methods

Methodology is largely based on that of Tulloss (2008); colours, including the colour of spores in deposit and other shades of white to cream (designated by letters A–G) are from Royal Botanic Garden, Edinburgh (1969) while codes for other colours are from Kornerup and Wanscher (1978). In the descriptions of basidiospores (and basidia) the notation [x/y/z] denotes x basidiospores measured from y basidiomes from z collections. Biometric variables for spores follow Tulloss (2012), i.e. ‘**L** = the average spore length computed for one specimen examined and the range of such averages, **L'** = average spore length computed for all spores measured, **W** = the average spore width computed for one specimen examined and the range of such averages, **W'** = average spore width computed for all spores measured, **Q** = the length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured, **Q** = the average value of **Q** computed for one specimen examined and the range of such averages, **Q'** = the average value of **Q** computed for all spores measured’.

DNA extraction, ITS amplification, cloning and sequence analysis follow the methodology in Davison *et al.* (2013). At least one cloned sequence for each species has been deposited in GenBank; sequence identifiers and voucher information are given under each species in this paper. The sequences were used as queries for NCBI nucleotide database using BLASTn (National Library of Medicine 2015).

Taxonomy

Amanita wadulawitu L.E.McGurk, E.M.Davison & E.L.J.Watkin, *sp. nov.* (MB 811810).

Type: City of Melville, Western Australia [precise locality withheld for conservation reasons], 14 May 2005, L. McGurk 2005 - 13 LM (*holo*: PERTH 8615861, ITS sequence GenBank JX398328).

Pileus 35–105 mm wide, to 16 mm thick, white or ivory white (B) aging cream (D) to vinaceous buff (6B2) to milky coffee (6D4), without surface staining or bruising, initially convex becoming plane with upturned margin; surface dry; margin non-striate, appendiculate when young. *Universal veil* on *pileus* adnate, felted or as small, soft pointed warts or as patches over whole disc, white. *Lamellae* adnate to adnexed, close to crowded, white to cream (B–C), 6–14 mm broad, the margin concolorous, fimbriate; *lamellulae* numerous in several lengths, shortest truncate, longest attenuate. *Stipe* 30–74 mm long, 10–36 mm wide, cylindrical or tapering upwards, white, surface smooth. *Partial veil* remnants superior or median or inferior, thin or thick with ragged margin, adpressed, sometimes separating

into circumsessile bands, fugacious or remaining as slight ridge, white. *Bulb* 30–80 × 17–42 mm, napiform or turbinate becoming fusiform or tapered, white. *Remains of universal veil at top of bulb* as ridges and warts or not apparent, white. *Pileus and stipe context* white becoming smoke grey (1B1) or vinaceous buff (6B2) in pileus and stipe base, in stipe solid becoming hollow. *Smell* none when young, of ammonia when old. *Spore deposit* white becoming cream (B) with age. (Figure 1)

Basidiospores [859/21/21] (8.5–)9–12.5(–15) × 4–5(–6) μm , (**L** = 10.2–12.5 μm ; **L'** = 11.0 μm ; **W** = 4.4–5.2 μm ; **W'** = 4.6 μm ; **Q** = (1.83–)2.00–2.80(–3.20); **Q** = 2.21–2.55; **Q'** = 2.38), colourless, thin-walled, smooth, amyloid, cylindric, occasionally elongate, the contents monoguttulate; apiculus sublateral, shortly cylindric, *c.* 1 × 2 μm rounded or truncate. *Pileipellis* to 200 μm thick in old specimens, colourless; filamentous hyphae 3–12 μm wide, colourless, with gelatinising walls, radially orientated with some interweaving; inflated cells not observed; vascular hyphae not observed; clamp connections not observed. *Pileus context* predominantly of filamentous hyphae 3–30 μm wide, with widest constricted at septa, thin-walled, colourless; acrophysalides to 200 × 35 μm clavate, colourless; vascular hyphae very infrequent, 4–8 μm wide pale yellow; clamp connections not observed. *Lamella trama* bilateral, divergent. *Central stratum* when well-hydrated comprising 18–25% of distance between bases of basidia on opposing hymenial surfaces, of thin-walled, colourless, filamentous hyphae 2–16 μm wide; inflated cells not observed; vascular hyphae not observed; clamp connections not observed. *Subhymenial base* with angle of divergence 35°–40° from central stratum with filamentous hyphae following smooth, broad curve to subhymenium, of dominant thin-walled, colourless, frequently

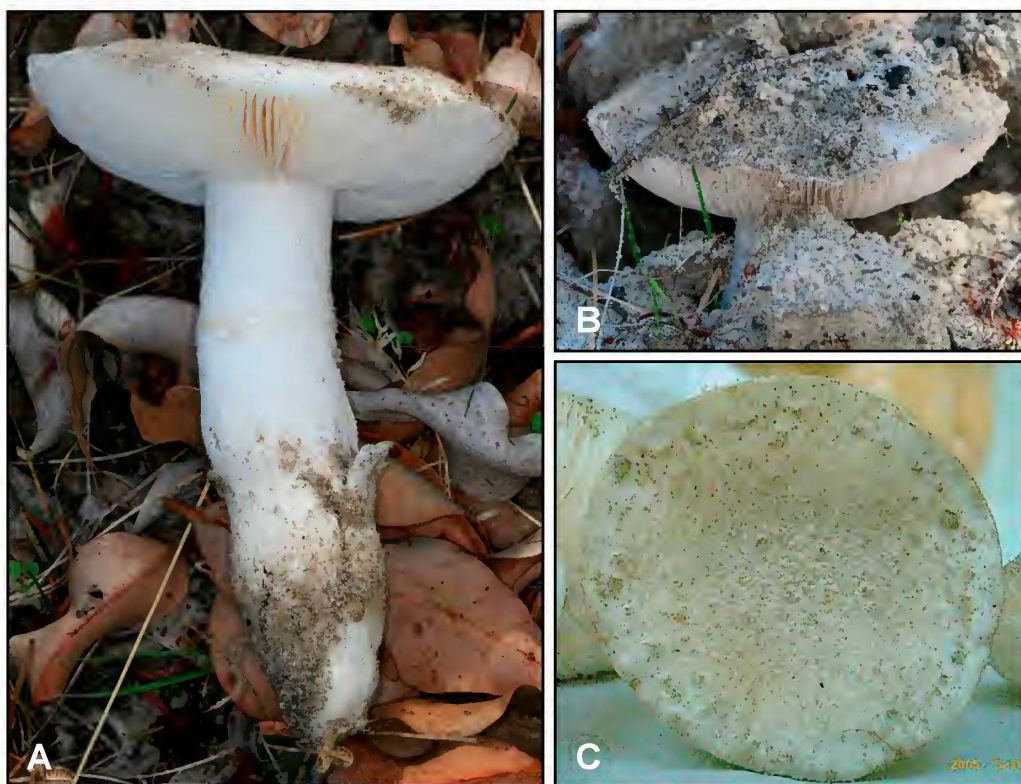


Figure 1. *Amanita wadulawitu*. A – mature basidiome, B – *in situ* showing emergence, fully formed, from sand, C – surface of pileus. Images from E.M. & P.J.N. Davison EMD 2-2008 (A–B) and L.E. McGurk 2005-13. © E.M. Davison (A, B) and L.E. McGurk (C).

branched filamentous hyphae 2–10 μm wide; inflated cells infrequent, colourless, to $80 \times 35 \mu\text{m}$, clavate, ventricose, cylindric or ovoid, terminal or intercalary; vascular hyphae infrequent, 2–3 μm wide, colourless; clamp connections not observed. *Subhymenium* with basidia arising terminally from barely inflated to inflated hyphal segments to 20 μm wide; clamp connections not observed. *Lamella edge tissue* sterile, with infrequent inflated cells cylindric or clavate, $15\text{--}30 \times 5\text{--}12 \mu\text{m}$, colourless, disarticulating, grouped; clamp connections not observed. *Basidia* [960/23/23] (31–)38–66 \times 9–13(–15) μm , thin-walled, colourless, c. 90% 4-spored, c. 10% 3-spored, occasionally 1-spored, sterigmata to $7 \times 2 \mu\text{m}$; clamp connections not observed. *Universal veil on pileus* not layered, with elements somewhat erect or irregularly disposed; filamentous hyphae 3–10 μm wide, colourless, gelatinising; inflated cells dominant, to $90 \times 65 \mu\text{m}$, ob/ovoid, globose, clavate or pyriform in terminal chains of up to 4 cells, colourless, gelatinising; vascular hyphae very infrequent, 5 μm wide, colourless; clamp connections not observed. *Universal veil on stipe base* without clear orientation; filamentous hyphae 5–15 μm wide, colourless, gelatinising; inflated cells dominant, to $85 \times 70 \mu\text{m}$, ellipsoid, ob/ovoid or spherical in terminal chains of up to 3 cells, colourless; vascular hyphae very infrequent, 5 μm wide, pale yellow; clamp connections not observed. *Stipe context* longitudinally acrophysalidic; filamentous hyphae 3–10 μm wide, colourless; acrophysalides dominant, to $470 \times 45 \mu\text{m}$, clavate, terminal or in intercalary chains, frequently anastomosed, colourless; vascular hyphae infrequent, 5–20 μm , pale yellow; clamp connections not observed. (Figure 2)

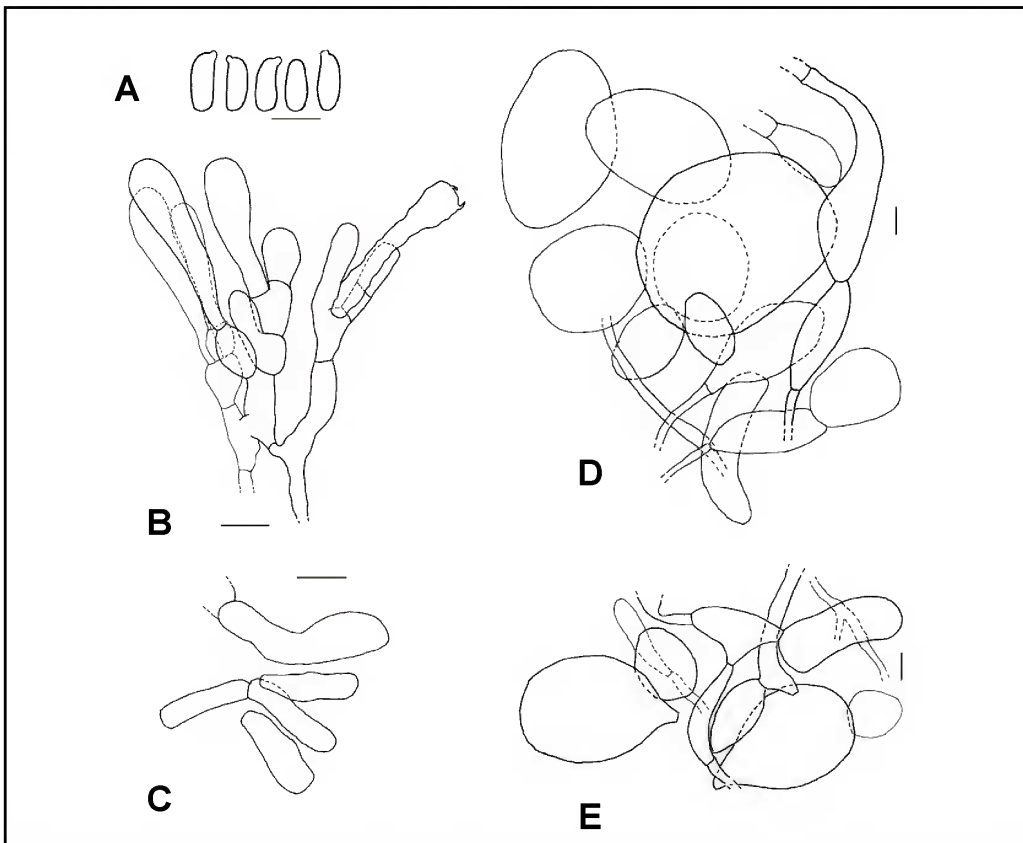


Figure 2. *Amanita wadulawitu*. A – spores from lamella; B – squash of basidia and subhymenium; C – lamella edge cells; D – section through universal veil from centre of pileus, unsquashed; E – universal veil from stipe base, squashed. Scale bars = 10 μm (A–C), 20 μm (D, E). Images from *E.M. & P.J.N. Davison EMD 1-2003*; (A), *E.M. & P.J.N. Davison EMD 1-1992* (B, D), *E.M. & P.J.N. Davison EMD 2-2008* (C) and *L.E. McGurk 2005-27* (E).

Diagnostic features. Small to large fruiting bodies with a white or ivory white pileus, which may age cream to milky coffee, and a white, adnate, universal veil that forms small, soft, pointed warts or patches over the whole disc. The gills are white to cream; the stipe is white with a bulb that is initially napiform or turbinate becoming fusiform or tapered. The fugacious white ring is superior, median or inferior; it sometimes splits into bands, and may develop a vinaceous buff margin with age. The flesh is white, aging grey. Old fruiting bodies have an ammoniac smell. The spores are amyloid and cylindric. The universal veil on the pileus has elements with no dominant orientation and is predominantly composed of inflated cells in short chains. Clamp connections are absent.

Other specimens examined. WESTERN AUSTRALIA: [localities withheld for conservation reasons] 12 May 1991, *E.M. Davison* 10/91 & *P.J.N. Davison* (PERTH); 29 Mar. 1992, *E.M. Davison* 1/92 & *P.J.N. Davison* (PERTH); 12 Apr. 1992, *E.M. Davison* 3/92 & *P.J.N. Davison* (PERTH); 10 May 1992, *E.M. Davison* 9/92 & *P.J.N. Davison* (PERTH); 3 May 2008, *E.M. Davison* 2-2008 & *P.J.N. Davison* (PERTH); 25 Apr. 2003, *E.M. Davison* 1-2003 & *P.J.N. Davison* (PERTH); 25 Apr. 2003, *E.M. Davison* 3-2003 & *P.J.N. Davison* (PERTH); 25 Apr. 2003, *E.M. Davison* 5-2003 (PERTH); 25 Apr. 2003, *E.M. Davison* EMD 6-2003 & *P.J.N. Davison* (PERTH); 14 May 2005, *L. McGurk* 2005-14 LM (PERTH); 14 May 2005, *L. McGurk* 2005-16 LM (PERTH); 20 May 2005, *L. McGurk* 2005-27 LM (PERTH); 20 May 2005, *L. McGurk* 2005-29 LM (PERTH).

Distribution and habitat. Solitary to gregarious in sandy soil, in native vegetation; nearby plants include *Corymbia calophylla*, *Eucalyptus marginata*, *E. todtiana*, *E. camaldulensis*, *Jacksonia furcellata*, *Banksia attenuata* and *B. menziesii*. Occurs in the Swan Coastal Plain SWA2 Perth IBRA subregion (Department of the Environment 2013).

Fruiting period. March to June.

Conservation status. To be listed as Priority Two under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (A. Jones pers. comm.).

Suggested common name. Long-spored *Lepidella*.

Etymology. A combination of *wadula* ‘long’ and *witu* ‘seed’ in the Western Australian Aboriginal Nhandia dialect of the Noongar language. A reference to the long spores of this taxon. The epithet is formed as a noun in apposition.

Affinities based on ITS sequence. The sequence JX398328 is 649 base pairs long. A BLASTn search showed that there are no matches in GenBank with an identity equal to or greater than 97%. The closest match is a species from the central west of Western Australia, *A. lesueurii* E.M.Davison clone 4 JX398315 (100% query coverage and 92% maximum identity).

Notes. The amyloid spores, non-striate appendiculate margin of the pileus when young, and floccose universal veil place this species in sect. *Lepidella*. On the pileus, the absence of rows of elongated cells and absence of a submembranous universal veil, place this in subsect. *Solitariae* Bas. The absence of clamps, the universal veil that is white and forms an initially felted layer over the pileus together with spores that are > 10 µm long place this species in stirps *Strobiliformis* (Bas 1969).

Two species from stirps *Strobiliformis* have been recently described from Western Australia (Davison *et al.* 2013). *Amanita wadulawitu* differs from *A. lesueurii* in its larger size, the flesh aging smoke

grey rather than pale vinaceous buff, and its cylindric spores ($Q = 2.21\text{--}2.55$ cf. elongate to cylindric ($Q = 1.90\text{--}2.26$) in *A. lesueurii*). *Amanita wadulawitu* differs from *A. wadjukiorum* E.M.Davison in the colour of the pileus and universal veil. Also the spores are longer; those of *A. wadjukiorum* are ellipsoid to elongate ($Q = 1.49\text{--}1.77$).

Amanita wadulawitu differs from other white or pale species that lack clamp connections within sect. *Lepidella* in Australia. The basidiomes are much larger than those of *A. angustispora* Cleland, and the universal veil differs because there is no free margin at the stipe base, and on the pileus it forms conspicuous soft warts not inconspicuous thin, felty remnants (Reid 1979). The spores of *A. wadulawitu* have a higher Q than *A. preisii* (Fr.) Sacc. (1.9–2.0) (Bas 1969), and *A. clelandii* E.-J. Gilbert (1.79–1.91) (Wood 1997).

Amanita kalamundae O.K.Mill., *Canad. J. Bot.* 69: 2697–2698 (1991) [as *A. kalamundi*] (MB560219).
Type: east of Kalamunda, Western Australia [precise locality withheld for conservation reasons], 18 June 1989, B. Dell OKM 23975 (*holo*: PERTH 02224283!).

Pileus 18–68 mm wide, to 5 mm thick, pale buff to pale vinaceous buff to clay buff to fawn to milky coffee (5B3–D5–6B3–C2–C3–D5–D6) in centre with margin paler, without surface staining or bruising, initially convex becoming plane; slightly tacky when moist; margin non-striate, appendiculate. *Universal veil on pileus* adnate, of small thin patches and scales mainly in centre, white becoming pale buff (5A3–B3). *Lamellae* free to adnexed to adnate, crowded to subdistant, white becoming cream (B) to pale clay pink (6B2), 3–10 mm broad; margin concolorous, fimbriate; *lamellulae* numerous, in several lengths, shortest truncate, longest attenuate. *Stipe* 35–48 mm long, 4–15 mm wide, cylindric or tapering upwards or flaring at apex, floccose below partial veil, white to cream (B). *Partial veil* apical to superior, descendant, flaring, soft, fugacious, white becoming cream (D–E) to pale saffron (5A4). *Bulb* 20–30 × 10–30 mm, turbinata becoming globose or ovoid or fusiform or tapered, white bruising straw (3A3). *Remains of universal veil* at top of bulb forming small free limb or warts, white. *Pileus and stipe context* white or cream (D) in pileus and stipe, slowly yellowing at stipe base, stipe solid, becoming hollow. *Smell* none when young, of chlorine or horseradish when old. *Spore deposit* white becoming cream (B) with age. (Figure 3)

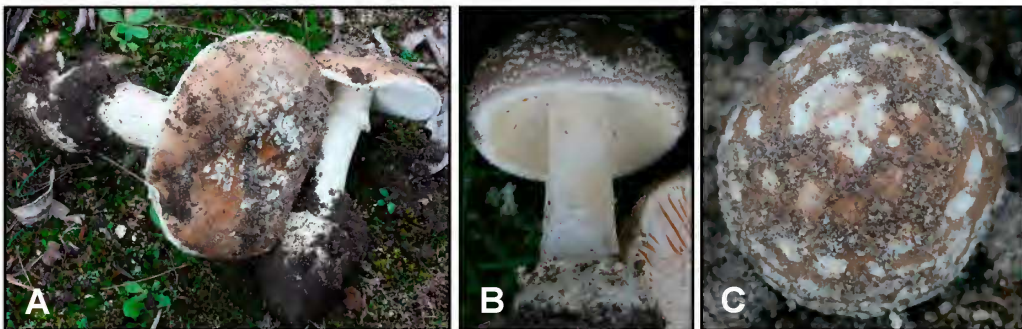


Figure 3. *Amanita kalamundae*. A – collection; B – young basidiome showing cream colouration on stipe and universal veil; C – universal veil on pileus. Images from N.L. Bougher & J. Keeble EMD 26-2013 (A) and E.M. & P.J.N. Davison EMD 28-2013 (B, C). © N.L. Bougher (A) and E.M. Davison (B, C).

Basidiospores [180/7/6] (7.5–)8.5–12.5(–13.5) × 5.0–7.5(–9.0) μm, (**L** = 8.9–11.1 μm; **L'** = 10.1 μm; **W** = 5.4–6.9 μm; **W'** = 6.4 μm; **Q** = (1.30–)1.36–1.91(–2.20); **Q** = 1.44–1.87; **Q'** = 1.61), colourless, thin-walled, smooth, amyloid, ellipsoid to elongate, occasionally cylindric, contents granular or monoguttulate; apiculus sublateral, shortly cylindric or tapered, *c.* 1 × 1 μm rounded or truncate. *Pileipellis* to 400 μm thick in old specimens, with a colourless gelatinised suprapellis to 200 μm thick, and pale brown subpellis; filamentous hyphae 2–8 μm wide, colourless, with thick gelatinising walls, radially orientated with some interweaving; inflated cells very infrequent, 10–15 μm wide; vascular hyphae infrequent, 2–5 μm wide, occasionally branched, pale yellow; clamp connections not observed. *Pileus context* filamentous hyphae dominant, 2–35 μm wide, with widest constricted at septa, thin-walled, colourless; acrophysalides to 250 × 60 μm thin-walled, clavate or ventricose, colourless; vascular hyphae very infrequent, 2–8 μm wide, occasionally branched, pale yellow; clamp connections 1 seen. *Lamella trama* bilateral, divergent. *Central stratum* when well-hydrated comprising 8–20% of distance between bases of basidia on opposing hymenial surfaces, of thin-walled, colourless, filamentous hyphae 2–10 μm wide; inflated cells not observed; vascular hyphae very infrequent, 2–5 μm, branches not observed, pale yellow; clamp connections very infrequent. *Subhymenial base* with angle of divergence 15°–30° from central stratum with filamentous hyphae following smooth, broad curve to subhymenium, of dominant thin-walled, colourless, frequently branched filamentous hyphae 3–20 μm wide, the widest constricted at septa; inflated cells infrequent, colourless, to 95 × 25 μm, clavate, ventricose, cylindric or strangulate, terminal or intercalary; vascular hyphae infrequent, 2–8 μm wide, occasionally branched, pale yellow; clamp connections very infrequent to frequent. *Subhymenium* with basidia arising terminally from barely inflated to pyriform hyphal segments to 15 μm wide; clamp connections infrequent. *Lamella edge tissue* sterile, with inflated cells infrequent to frequent, broadly clavate, pyriform, clavate, ovoid, ventricose or capitate, 20–80 × 10–25 μm, colourless, disarticulating; clamp connections infrequent. *Basidia* [160/7/6] (38–)40–59(–70) × (9–)10–13(–14) μm, thin-walled, colourless, *c.* 98% 4-spored, *c.* 2% 3-spored, occasionally 1-spored, sterigmata to 7 × 2 μm; clamp connections frequent. *Universal veil on pileus* not layered, with elements somewhat erect; filamentous hyphae 2–10 μm wide, colourless, gelatinising; inflated cells dominant, ovoid (to 60 × 40 μm), spherical (to 40 × 40 μm), pyriform (to 80 × 35 μm), ellipsoid (to 45 × 35 μm) or clavate (to 35 × 12 μm), terminal or occasionally in terminal chains of 2 cells, pale yellow, gelatinising; vascular hyphae not observed; clamp connections not observed. *Universal veil on stipe base* without clear orientation; filamentous hyphae 2–12 μm wide, colourless, gelatinising; inflated cells dominant or equal, colourless, ovoid (to 60 × 30 μm), clavate (to 100 × 40 μm), pyriform (to 55 × 35 μm), spherical (to 50 × 50 μm), ellipsoid (to 25 × 15 μm) or turbinate (to 50 × 30 μm), terminal or occasionally in terminal chains of 2 cells, pale yellow, gelatinising; vascular hyphae very infrequent, 3 μm, pale yellow; clamp connections 1 seen. *Stipe context* longitudinally acrophysalidic; filamentous hyphae 3–13 μm wide, colourless; acrophysalides dominant, to 400 × 50 μm, clavate, terminal occasionally intercalary, colourless, gelatinising; vascular hyphae frequent to infrequent, 2–20 μm, occasionally branched, pale yellow; clamp connections not observed. *Partial veil* not examined. (Figure 4)

Diagnostic features. Very small to medium-sized fruiting bodies with a buff to milky coffee pileus with a pale margin, and a universal veil of small thin patches and scales that is white or pale buff. The gills are white to cream or pale clay pink; the stipe is white to cream with a superior partial veil that is initially white and becomes cream to pale saffron with age. The bulb is ovoid or fusiform, it is white but bruises yellow; the universal veil remains at the top of the bulb as a small white free limb or as warts. The flesh at the stipe base is white, yellowing slowly. When old, the fruiting bodies smell strongly of chlorine. The spores are amyloid and ellipsoid to elongate; the universal veil on the pileus has elements that are somewhat erect in the centre of the pileus and is composed of dominant, terminal, inflated cells. Clamp connections are present in the gills and at the base of basidia.

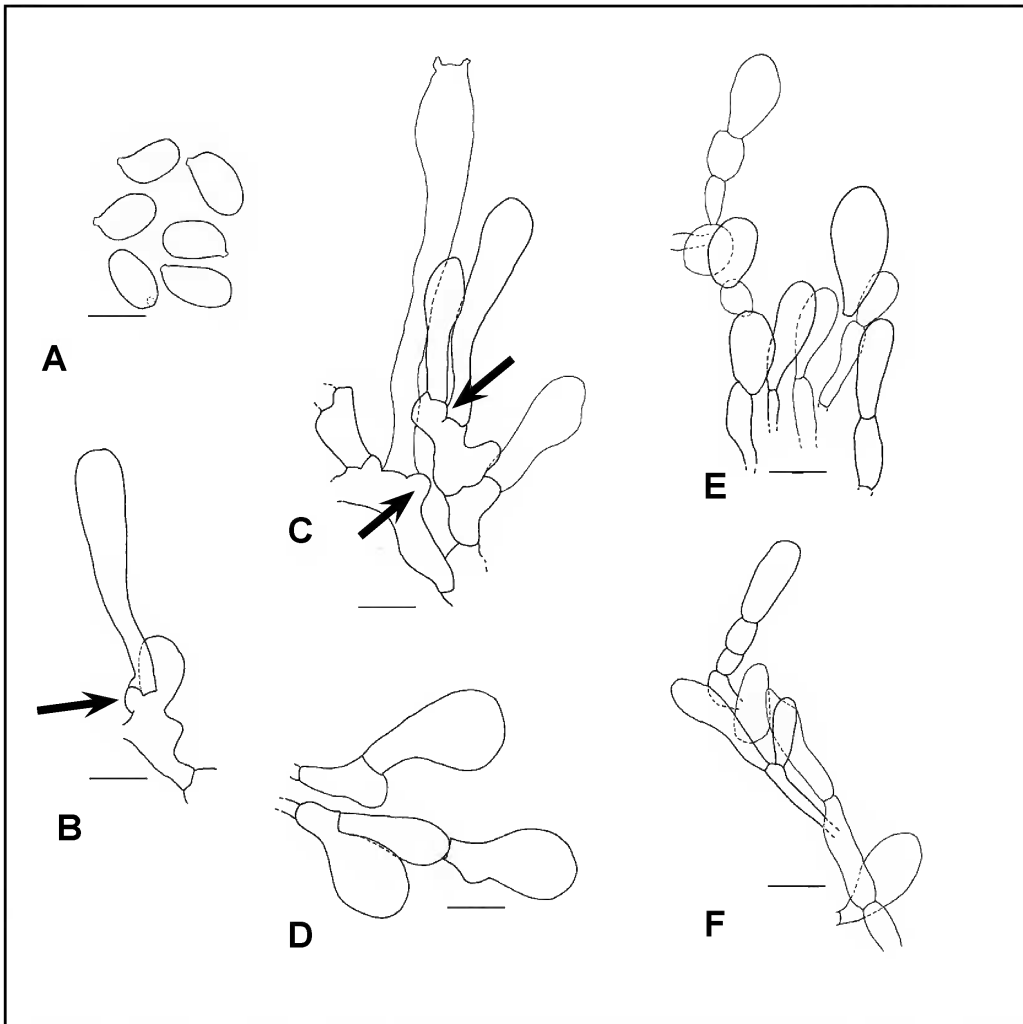


Figure 4. *Amanita kalamunda*. A – spores from lamella; B & C – squash of basidia and subhymenium, clamp connections indicated with arrows; D – lamella edge cells; E – section through universal veil from centre of pileus, unsquashed; F – universal veil from stipe base, squashed. Scale bars = 10 μm (A–D), 40 μm (E, F). Images from R.M. Robinson, R.H. Smith & K. Syme FC 891 (A, C), B. Dell OKM 23975 (B) and N.L. Bougher & J. Keeble EMD 26-2013 (D–F).

Other specimens examined. WESTERN AUSTRALIA [localities withheld for conservation reasons]: 2 June 2013, N.L. Bougher & J. Keeble EMD 26-2013 (PERTH, GenBank accession KP898376-82); 2 June 2013, E.M. Davison 28-2013 & P.J.N. Davison (PERTH); 22 June 2005, R.M. Robinson, R.H. Smith & K. Syme FC 891 (PERTH); 31 May 2003, K. Syme EMD 8-2003 (PERTH, GenBank accession JX398319); 28 June 1995, I.C. Tommerup, B. Dunstan & N.L. Bougher s.n. (PERTH).

Distribution and habitat. Gregarious in sandy clay or lateritic clay soil, in native vegetation; nearby plants include *Taxandria linearifolia*, *Allocasuarina fraseriana*, *Bossiaea ornata*, *Eucalyptus marginata*, *E. diversicolor*, *E. patens*, *E. rudis*, *E. wandoo*, *Macrozamia riedlei*, *Banksia grandis* and *Hakea lissocarpha*. Occurs in the Northern Jarrah Forest, Southern Jarrah Forest and Avon Wheatbelt IBRA subregions (Department of the Environment 2013).

Fruiting period. May to June.

Conservation status. To be listed as Priority Three under Department of Parks and Wildlife Conservation Codes for Western Australian Flora (A. Jones pers. comm.).

Suggested common name. Kalamunda *Lepidella*.

Affinities based on ITS sequence. The sequence JX398319 (PERTH 08616019) is 679 base pairs long and sequences KP898376-82 (PERTH 08615993) are between 712 and 719 base pairs long. Aligning these sequences shows that there is an 11 base pair indel (CTTTCTTTCCT) in JX398319 in ITS 1 that is not present in sequences KP898376-82. Hughes *et al.* (2009) suggest that such multibase indels should be considered as the result of a single event. When this is done, these sequences show between 0.30% and 2.38% divergence (Table 1), i.e. within the 3% divergence indicative of conspecificity (Hughes *et al.* 2009). The next closest matches are KP137085 and KP137086 (AD-C55022 clones 12.3 and 12.5), from an undescribed species from South Australia, with 100% coverage and between 3.12% and 4.46% divergence from *A. kalamundae* (Table 1).

Table 1. Comparison of the percent divergence of cloned sequences of *Amanita kalamundae* derived from PERTH 08616019 (JX398319), PERTH 08615993 (KP898376-82) (in bold) and AD-C55022 (KP137085-6). The 11 base pair indel in JX398319 has been treated as a single event.

	JX398319	KP898376	KP898377	KP898378	KP898379	KP898380	KP898381	KP898382	KP137085	KP137086
KP898376	1.04									
KP898377	1.34	0.59								
KP898378	0.74	0.59	0.89							
KP898379	2.38	1.63	1.63	1.93						
KP898380	1.34	0.30	0.59	0.89	1.34					
KP898381	1.04	0.89	1.19	0.59	2.23	1.19				
KP898382	1.34	0.30	0.59	0.89	1.63	0.30	1.19			
KP137085	3.57	3.57	3.27	3.42	3.12	3.86	3.71	3.86		
KP137086	3.27	3.42	3.42	3.12	4.46	3.71	3.42	3.71	2.67	

Notes. Miller (1991) named this species after Kalamunda, the place where the type was collected; however, the original epithet was misspelt and this has been corrected in MycoBank and *Index Fungorum* to *kalamundae* (Articles 60.7 and 60.12, International Code of Nomenclature (McNeill *et al.* 2012)). The species was described from a single collection. The additional collections used to expand Miller's observations here fit well with his field description. Re-examination of the type, as well as these additional collections, shows that clamp connections are present in the lamellae, and are frequent at the base of basidia. These were not mentioned by Miller (1991).

The presence of amyloid spores and the appendiculate margin of the pileus suggest that the best placement for *A. kalamundae* is in subg. *Lepidella* sect. *Lepidella sensu* Bas (1969) rather than sect. *Validae* (Fr.) Singer as suggested by Miller (1991). The structure of the universal veil places it in subsect. *Solitariae*. The presence of clamp connections, the universal veil composed of elements which are erect in the centre of the pileus, are composed of inflated cells intermixed with hyphae, and with inflated cells present at the base of the wart indicates that *A. kalamundae* is in stirps *Microlepis* (Bas 1969).

Amanita kalamundae is similar to *A. pyramidiferinus* A.E.Wood from New South Wales, but differs in the darker colour of the fugacious partial veil, yellowing context, and chlorine smell.

Acknowledgements

LEM was supported by a Curtin University Postgraduate Scholarship. This work was supported by Australian Biological Resources Study grants CN211-40 and CN213-05. We thank R.M. Robinson for information about a collection (PERTH 6658903), and T.W. May for clarification of the epithet *kalamundae* and for his constructive comments towards improvement of this manuscript. Associate Professor Elder in Residence Simon Forrest is thanked for cultural and linguistic advice in naming *A. wadulawitu*. The editorial staff of *Nytsia* are thanked for their advice.

References

- Bas, C. (1969). Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. *Persoonia* 5: 285–579.
- Corner, E.J.H. & Bas, C. (1962). The genus *Amanita* in Singapore and Malaya. *Persoonia* 2: 241–304.
- Davison, E.M., McGurk, L.E., Bougher, N.L., Syme, K. & Watkin, E.L.J. (2013). *Amanita Iesueurii* and *A. wadjukiorum* (Basidiomycota), two new species from Western Australia, and an expanded description of *A. fibrillopes*. *Nytsia* 23: 589–606.
- Department of the Environment (2013). *Australia's bioregions (IBRA)*, IBRA7, Commonwealth of Australia. <http://www.environment.gov.au/land/nrs/science/ibra#ibra> [accessed 2 January 2014].
- Grgurinovic, C.A. (1997). *Larger fungi of South Australia*. (The Botanic Gardens of Adelaide and State Herbarium: Adelaide.)
- Hughes, K.W., Petersen, R.H. & Lickey, E.B. (2009). Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. *New Phytologist* 182: 795–798.
- McNeill, J., Barrie, F.R., Buch, W.R., Demoulin, V., Greuter, W., Hawkesworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'Homme van Reine, W.F., Smith, G.F. & Wiersma, J.H. (2012). *International Code of Nomenclature for algae, fungi, and plants*. (Koeltz Scientific Books: Königstein, Germany.)
- Kornerup, A. & Wanscher, J.H. (1978). *Methuen handbook of colour*. (Methuen: London.)
- Miller, O.K. (1991). New species of *Amanita* from Western Australia. *Canadian Journal of Botany* 69: 2692–2703.
- Miller, O.K. (1992). Three new species of *Amanita* from Western Australia. *Mycologia* 84: 679–686.
- National Library of Medicine (2015). Blast®. http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome [accessed 3 March 2015].
- Reid, D.A. (1980). A monograph of the Australian species of *Amanita* Pers. ex Hook. (Fungi). *Australian Journal of Botany, Supplementary Series* No. 8: 1–97.
- Royal Botanic Garden, Edinburgh (1969). *Flora of British fungi: colour identification chart*. (Her Majesty's Stationery Office: Edinburgh.)
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. & Fungal Barcoding Consortium (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109: 6241–6246.
- Tulloss, R.E. (2008). Notes on methodology for study of *Amanita* (Agaricales). In: Tulloss, R.E. & Yang, Z.L. *Studies in the genus Amanita Pers. (Agaricales, Fungi)*. <http://pluto.njcc.com/~ret/amanita/mainaman.html> [accessed 1 August 2009].
- Tulloss, R.E. (2012). Biometric variables: meanings and how to define a range. <http://www.amanitaceae.org/?How%20to%27s> [accessed 26 March 2013].
- Wood, A.E. (1997). Studies in the genus *Amanita* (Agaricales) in Australia. *Australian Systematic Botany* 10: 723–854.